SKIN COSMETIC COMPOSITION CONTAINING KIDNEY BEAN EXTRACTS

Field of the Invention

The present invention is related to a skin cosmetic composition containing kidney bean extracts.

Background of the Invention

In cosmetics-related field, the most critical task in preventing skin aging is suppression of wrinkle formation or improvement of wrinkle. Female hormone, estrogen stimulates fibroblast in dermis to accelerate collagen synthesis and metabolism, as well as to stimulate synthesis of hyaluronic acid, which is very important for skin moisture retention. In addition, estrogen helps proliferation of basal layer cell in epidermis, which makes epidermis thicker to reinforce skin cell tissue. Further, estrogen reduces the formation of sebum by inhibiting sebaceous gland growth through control of sebaceous gland, and keeps hair thin and short by reduction of growth rate thereof. In particular, estrogen is involved in collagen metabolism, inhibits over-glycation of collagen, induces post-translational modification, and inhibits degradation of collagen by regulating the expression of collagenase, the degradation enzyme of collagen. As such, major function of estrogen on skin is to reinforce skin action and to keep skin smooth, elastic and soft.

However, as one grows older, estrogen-secreting function of the genital organ declines, and around menopause period when the production

function is lost, the formation of estrogen is stopped. When hormone balance is lost by the stop of estrogen formation, because of the non-competitive effect of male hormone, testosterone, endocrine aging with estrogen deficiency is stimulated. The endocrine aging is a type of physiological skin aging, which occurs at menopause period.

Estrogen deficiency causes skin changes, and a typical phenomenon is skin drying. Because of estrogen deficiency, formation of collagen and elastic fiber is suppressed in fibroblast, which leads to thinner skin and reduction of skin elasticity. Additionally, reduction in synthesis of hyaluronic acid results in weakening of moisture retention function of skin. Further, cross-linking of collagen increases due to increase in collagen glycation, resulting in skin hardening and elasticity reduction.

Studies on a method for inhibiting skin aging due to estrogen deficiency have been reported. Among these, hormone replacement therapy involves direct supply of estrogen. The direct supply of estrogen brought remarkable effects such as increase in collagen synthesis, hyaluronic acid synthesis and epidermis cell proliferation leading to an increase in epidermis thickness.

However, as it is not allowed to use estrogen itself in cosmetics, alternative methods have been sought. In cosmetic field, instead of direct method for estrogen deficiency, indirect method using general inhibitory substance against skin aging such as moisturizing substance, anti-aging substance, free radical eliminating substance or UV blocking agent.

Summary of the Invention

To improve female skin where aging is rapidly progressed around menopause period, it is important to develop a substance having function similar to estrogen. And as for the estrogen-like substance, the functions of collagen synthesis and hyaluronic acid synthesis are required, from the viewpoint of cosmetics to inhibit skin aging.

The inventors of the present invention have studied on wild herb medicine to develop estrogen-like substance, and found that kidney bean extract shows a superior effect on cell proliferation, collagen synthesis and hyaluronic acid synthesis, and completed the present invention.

Detailed Description of the Invention

The present invention provides a skin cosmetic composition containing kidney bean extracts.

In the cosmetic composition of the present invention, kidney bean extracts is preferably contained in an amount of 0.05 to 10.0% by weight based on dried weight of the cosmetic composition.

Kidney bean belongs to Fabaceae family, and includes vine kidney bean (Phaseolus vulgaris L.), red kidney bean (Phaseolus multiflorus WILD), kidney bean (Phaseolus vulgaris L. var. humilis ALEF) and white kidney bean (Phaseolus multiflorus WILD for albus BAIKEY), and these all can be used in the present invention.

The kidney bean extracts according to the present invention are prepared as follows. Firstly, kidney bean seeds are dried, washed with purified water, dried and pulverized. As extraction solvent, water, lower

alcohol (e.g. methanol, ethanol), mixture of water and lower alcohol, acetone, ethyl acetate, 1,3-butylene glycol, hexane, diethyl ether, n-propanol, isopropanol, or n-butanol is added to 1 to 15 times of dried weight of the pulverized kidney bean, and deposited for 1 to 15 days at an ambient temperature to extract active ingredient. Thus extracted solution was subjected to vacuum concentration in a distillation apparatus with cooling condenser to form kidney bean extracts.

The kidney bean extracts of the present invention may be contained in various cosmetics such as basal cosmetics (e.g., softener, cream, essence, cleansing foam, cleansing water, pack and body oil), color cosmetics (e.g., foundation, lipstick, mascara and make up base), and hair cosmetics (e.g., shampoo, hair conditioner and hair gel) can be enumerated.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of this invention in any manner.

Example 1

Kidney bean was washed with purified water, dried and pulverized to obtain kidney bean powder. 1 kg of kidney bean powder was then mixed with 5 L of water, and extracted for 5 days at ambient temperature. The extracts were filtered with 300-mesh filter cloth, and were left for 10 days at the temperature of 4-15°C (i.e. low temperature maturation), and then filtered with Whatman No. 2 filter paper. These extracts were subjected to vacuum concentration at 70°C in a distillation apparatus with

condenser, and dried to obtain 132.20 g (dried weight) of kidney bean extracts. Extraction yield was 13.2±0.8%.

Example 2

1 kg of kidney bean powder which was obtained by washing kidney bean with purified water, drying and pulverization, was mixed with 5 L of 10% ethanol, and then extracted for 3 days in extractor with condenser. The extracts were filtered with 300-mesh filter cloth, and maturated for 10 days at 4 to 15°C, and then filtered with Whatman No. 2 filter paper. These extracts were subjected to vacuum concentration at 70°C in a distillation apparatus with condenser, and dried to obtain 127.4g (dried weight) of kidney bean extracts. Extraction yield was 127±0.7%.

Examples 3 to 21

In each example, extraction was performed according to Example 2, except for using the solvent in Table 1. Each extraction yield was shown in Table 1.

Table 1

Example	Solvent	Extraction yield (% average ± standard deviation)
Example 3	20% ethanol	12.1 ± 0.7
Example 4	30% ethanol	11.2 ± 0.6
Example 5	40% ethanol	9.9 ± 0.4

Example 6	50% ethanol	8.4 ± 1.0
Example 7	60% ethanol	6.7 ± 0.5
Example 8	70% ethanol	5.4 ± 0.3
Example 9	80% ethanol	4.3 ± 0.3
Example 10	90% ethanol	2.5 ± 0.1
Example 11	100% ethanol	1.7 ± 0.1
Example 12	80% methanol	4.2 ± 0.4
Example 13	100% methanol	1.5 ± 0.1
Example 14	Acetone	0.7 ± 0.1
Example 15	Ethyl acetate	1.1 ± 0.2
Example 16	50% 1,3-butylene glycol aqueous solution	5.8 ± 0.5
Example 17	Hexane	0.8 ± 0.1
Example 18	Diethyl ether	0.3 ± 0.1
Example 19	n-propanol	0.2 ± 0.1
Example 20	Iso-propanol	0.2 ± 0.1
Example 21	n-butanol	0.2 ± 0.1

Experimental Example 1

Cell proliferation effect of kidney bean extracts

1) Experimental method

Human normal fibroblast was inoculated in each well of 96-well microplate ($1x10^4$ cell/well) and cultivated in DMEM medium for 24 hours. After the culture, culture medium was changed to serum-free DMEM

medium containing 250 μg/ml of kidney bean extracts prepared in Examples 1 to 21, and cultivated further for 24 hours. MTT solution [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide: 5 mg/ml] 10 μl was added, and after 4 hours, medium was removed. 100 μl of dimethyl sulfoxide solution was added to each well and stirred for 20 minutes. Absorbance was measured at 570 nm using microplate reader. Same procedure was repeated for three times.

2) Experimental result

Cell proliferation effect was calculated by following equation and its result was shown in Table 2.

Cell proliferation effect (%) = {(Absorbance of extracts – Absorbance of control)/(Absorbance of control)} x 100

Table 2

Example No.	Cell proliferation effect (% by weight)
Example 1	15.4 ± 1.3
Example 2	18.6 ± 1.6
Example 3	21.5 ± 2.5
Example 4	27.6 ± 0.9
Example 5	35.9 ±1.3
Example 6	42.7 ± 2.1
Example 7	51.3 ± 2.4

Example 8	58.7 ± 2.5
Example 9	54.6 ± 2.5
Example 10	56.5 ± 2.3
Example 11	52.8 ± 2.0
Example 12	54.5 ±2.2
Example 13	45.7 ±1.6
Example 14	31.4 ± 1.5
Example 15	44.8 ± 1.8
Example 16	52.1 ± 2.4
Example 17	10.3 ± 0.6
Example 18	11.4 ± 0.8
Example 19	22.4 ± 1.4
Example 20	25.3 ± 1.6
Example 21	21.7 ± 1.4

* The above values are average of three times experiments.

The above result clearly shows that kidney bean extracts of the present invention exhibits superior effect on cell proliferation.

Experimental example 2

Effect of kidney bean extracts on collagen synthesis

1) Experimental method

Human normal fibroblast was inoculated to 96-well microplate ($2x10^4$ cell/well) and cultivated for 24 hours. After the culture, culture medium was changed to serum-free DMEM medium containing kidney

bean extracts in the following Table 3, and cultivated for 48 hours. In Table 3, control group was cultivated without kidney bean extracts. Kidney bean extracts prepared in Example 8 was used. Before 24 hours from the end of culture, ascorbic acid (50 μg/ml) was added to stimulate collagen synthesis. After the culture, each well was washed and changed to serum-free DMEM medium and cultivated for another 24 hours. Supernatants of each well was collected and amount of procollagen type IC-peptide (PICP) was measured by using kit (Takara, Kyoto, Japan) and the amount of PICP was converted into ng/2x10^{4}cell.

2) Experimental result

Kidney bean extracts of the present invention exhibited effect on synthesis of collagen I in human normal fibroblast and the result was given in Table 3.

Table 3

Concentration	Amount of collagen synthesis (ng/2x10^{4}cell)
Control	151
100 μg/ml	204
250 μg/ml	254
500 μg/ml	291

According to the above result, synthesis yield of collagen increases as the concentration of kidney bean extracts increases, showing that kidney

bean extracts according to the present invention exhibits superior effect of increasing collagen synthesis.

Experimental example 3

Effects of kidney bean extracts on cell proliferation

Experiment was carried out according to the procedure of Experimental example 1. Kidney bean extracts prepared in Example 8 was used in different concentration. The result is as described in Table 4.

Table 4

Concentration	Absorbance (570 nm)	Cell proliferation effect (%)
Control	0.320 ± 0.008	-
100 μg/ml	0.414 ± 0.012	29.4
250 μg/ml	0.454 ± 0.030	41.6
500 μg/ml	0.563 ± 0.015	75.9

The above result shows that cell proliferation effect increases as the concentration of kidney bean extracts increases, and based on this, it can be seen that kidney bean extracts of the present invention exhibits superior effect of cell proliferation.

Experimental example 4

Effect of kidney bean extracts on hyaluronic acid synthesis

Human normal fibroblast was inoculated to 6-well microplate ($3x10^4$ cell/well) and cultivated for 48 hours. At this time, as culture

medium, DMEM medium containing 10% fetal calf serum, penicillin and streptomycin (100 μg/ml) was used. After the culture, medium was changed to a culture medium containing various concentrations of kidney bean extracts as in Table 3 and cultivated for 24 hours. Kidney bean extracts prepared in Example 8 was used. After the culture, only medium part was collected, and then trichloroacetic acid was added to final concentration of 10% and stored at 4° C for 24 hours in order to remove protein. This solution was centrifuged, its supernatant was subjected to dialysis, it was treated with NaCl to a final concentration of 0.04 M, and then cetylpyridium chloride was added to precipitate hyaluronic acid. After centrifugation, the precipitate was dissolved in 4M NaCl, reprecipitated with ethanol, and this precipitate was dissolved in purified water. For quantitative analysis of hyaluronic acid, electrophoresis was conducted by using cellulose acetate strip [Hata, R. & Nagai, Y. (1972): Anal. Biochem. 45, pp 462-468].

After the electrophoresis, it was soaked in 3% acetic acid containing 0.5% Alcian blue, washed, dried. Then the amount of hyaluronic acid was measured by 600 nm scanner. The result is shown in Table 5.

Table 5

Concentration	Hyaluronic acid synthesis increase effect (%)
100 μg/ml	35.8
500 μg/ml	56.4

From the above result, it can be seen that synthesis of hyaluronic acid in normal fibroblast increases as the concentration of kidney bean extracts increases. Therefore, it could be seen that kidney bean extracts exhibits effect of increasing hyaluronic acid synthesis.

The followings are formulation examples of cosmetic composition containing kidney bean extracts of the present invention. They were prepared by the conventional methods in cosmetics field.

Formulation 1: skin softener

Skin softener containing kidney bean extracts was prepared by using ingredients and amount described in the following Table 6.

Table 6

Ingredient	Content (% by weight)
Kidney bean extract	5.0
1,3-butylene glycol	6.0
Sodium hyaluronate	2.0
Glycerin	4.0
PEG 4000	1.0
Polysorbate 20	0.5
Ethanol	10.0
Preservative	q. s.
Benzophenone-9	0.05

Flavor Purified water	Adequate amount q. s.
Total	100

Formulation 2: Milk lotion

Milk lotion containing kidney bean extracts was prepared by using the ingredient and amount listed in the following Table 7.

Table 7

Ingredient	Content (% by weight)
Kidney bean extract	5.0
Stearic acid	0.4
1,3-butylene glycol	6.0
Ccetostearyl alcohol	1.2
Glycerin	4.0
Glyceryl stearate	1.0
Triethanol amine	0.25
Tocopheryl acetate	3.0
Liquid paraffin	5.0
Squalene	3.0
Macadamia nut oil	2.0
Polysorbate 60	1.5
Sorbitan sesquioleate	0.6
Carboxyvinyl polymer	0.15

Preservative	q. s.
Flavor	q. s.
Purified water	q. s.
Total	100

Formulation 3: nutrition cream

Nutrition cream containing kidney bean extracts was prepared by using the ingredient and amount listed in the following Table 8.

Table 8

Ingredient	Content (% by weight)
Kidney bean extract	5.0
Vaseline	7.0
Cetostraryl alcohol	2.5
Gylceryl stearate	2.0
Stearic acid	1.5
Liquid paraffin	10.0
Bess wax	2.0
Polysorbate 60	1.5
Sorbitan cesquioleate	0.8
Squalane	3.0
1,3-butylene glycol	6.0
Glycerine	4.0
Triethanolamine	0.5

Tocoperyl acetate	0.1
Preservative	q. s.
Flavor	q. s.
Purified water	q. s.
Total	100

Formulation 4: Essence

Essence containing kidney bean extracts was prepared by using the ingredients and amount described in the following Table 9.

Table 9

Ingredient	Content (% by weight)
Kidney bean extracts	5.0
Glycerin	10.0
PEG 1500	2.0
Alantoin	0.1
Panthenol	0.3
EDTA	0.02
Benzophenone-9	0.04
Hydroxyethyl cellulose	0.1
Sodium hyaluronate	8.0
Carboxyvinyl polymer	0.2
Triethanolamine	0.18
Octyldodeces-25	0.6

Ethanol	6.0
Preservative, Flavor, Coloring agent	Very small amount
Purified water	q. s.
Total	100

Formulation 5: Massage cream

Massage cream containing kidney bean extracts was prepared by using ingredients and amount described in the following Table 10.

Table 10

Ingredient	Content (% by weight)
Kidney bean extracts	3.0
Glyceryl stearate	2.0
Cetostearyl alcohol	2.5
Stearic acid	1.0
Polysorbate 60	1.5
Sorbitan stearate	0.6
Isostearyl isostearate	5.0
Squalene	5.0
Mineral oil	35.0
Dimethicon	1.0
Xantan gum	0.1
Hydroxyethyl cellulose	0.12
Glycerin	6.0

Triethanolamine	0.5
Preservative, flavor, coloring agent	q. s.
Purified water	q. s.
Total	100

Formulation 6: Pack

Pack containing kidney bean extracts was prepared by using the ingredients and amount given in the following Table 11.

Table 11

Ingredient	Content (% by weight)
Kidney bean extracts	3.0
Polyvinyl alcohol	15.0
Cellulose gum	0.15
Glycerin	3.0
PEG 1500	2.0
Panthenol	0.4
Alantoin	0.1
Ethanol	6.0
PEG 40 hydrogenated castor oil	0.3
Preservative, flavor, coloring agent	Very small amount
Purified water	q. s.
Total	100